REMARKS

The undersigned attorney wishes to thank the Examiner for the courtesies extended during the Interviews. As the Examiner agreed during the Interviews, the amendments to the claims and specification presented above place the application in condition for allowance.

We note that the filing date for the above-referenced application as recited in the Office Action (*i.e.*, July 15, 1997) is incorrect. (Paper No. 35). The correct filing date is July 14, 1997, as evidenced by a copy of the Corrected Filing Receipt attached hereto as Exhibit 4.

As requested by the Examiner, the specification has been amended to insert a paragraph reciting the current address of the American Type Culture Collection (ATCC).

As requested by the Examiner, the specification has further been amended to replace the sections entitled "Appendices for United States Letters Patent" and "Tables for United States Letters Patent" with substitute sections that include pages containing Appendix I, and Tables 4, 6, and 7.

As requested by the Examiner, claims 1-4 have been amended to recite that the lysine-utilizing DAPA aminotransferase is a --*Bacillus subtilis*-- lysine-utilizing DAPA aminotransferase. Support for this amendment is found in the specification at, for example, page 4, ln. 24 to page 5, ln. 5 and page 10, lns. 1-6.

As further requested by the Examiner and for the sake of clarity, claims 11 and 21 have been amended to replace the recitation of "the *bioA* gene" with --a

polynucleotide encoding a DAPA aminotransferase-- and to insert a comma after the terms "step" and "aminotransferase." Support for this amendment is found in the specification at, for example, page 2, lns. 1-2 and page 3, lns. 9-10.

It is submitted that no new matter has been introduced by the foregoing amendments. Approval and entry of the amendments is respectfully solicited.

Objections to the Specification

The Examiner objected to the Specification. (Paper No. 35 at 2). In making the objection, the Examiner asserted that the previously submitted amendment "could not be entered since the location provided by Applicants in regard to the insertion of the paragraph is not consistent with what is in page 8" and further that "the address of the American Type Culture Collection is incorrect. The new address is 10801 University Boulevard, Manassas, VA 20110-2209." (*Id.*).

With a view towards furthering prosecution and in accordance with the Examiner's request, the specification has been amended in the manner requested by the Examiner. Accordingly, this objection is rendered moot and should be withdrawn.

The Examiner further asserted that "parts of Appendix I, Table 4, 6, and 7 are not legible. ... Applicants are requested to submit a copy of such Appendix and Tables with the appropriate margins to avoid perforation of text." (Paper No. 35 at 3).

The specification has been amended as set forth above to include pages containing Appendix I, and Tables 4, 6, and 7 that have been formatted so that the text

will not be obscured when the PTO punches these pages. Accordingly, this objection is rendered moot and should be withdrawn.

Objections to the Claims

Claim 11 was objected to for containing "informalities." (Paper No. 35 at 3). In making the objection, the Examiner suggested that "commas be inserted immediately after the term 'step' and immediately after the term 'gene'." (*Id.*).

With a view towards furthering prosecution and in accordance with the Examiner's request, claim 11 has been amended as set forth above. Accordingly, this objection is rendered moot and should be withdrawn.

§112, Second Paragraph Rejections

Claim 11 was rejected under 35 U.S.C. § 112, second paragraph. (Paper No. 35 at 4). In making the rejection, the Examiner asserted that "[c]laim 11 is indefinite in the recitation of 'bioA gene is deregulated in said bacterium'.... It is suggested that the claim be either amended to clearly indicate the organism associated with the specific gene designation or amended to indicate the gene product encoded." (*Id.* at 4-5).

With a view towards furthering prosecution and as suggested by the Examiner, claim 11 has been amended to replace the recitation of "bioA" with --a polynucleotide encoding a DAPA aminotransferase--. Accordingly, this rejection is rendered moot and should be withdrawn.

§112, First Paragraph Rejections

1. Written Description

Claims 1-22 were rejected under 35 U.S.C. § 112, first paragraph. (Paper No. 35 at 5). In making the rejection, the Examiner asserted that:

> The claims are directed to a method wherein the bacterium comprises any lysine-utilizing DAPA aminotransferase, and while the specification discloses 6 strains which have been deposited, the strains deposited contain *B. subtilis* lysine utilizing aminotransferase only. Therefore, ... it is unclear as to how one of skill in the art can conclude that the method claimed is adequately described. (*Id.* at 7).

The Examiner further indicated that "[t]he instant rejection may be overcome by limiting the claims to a *B. subtilis* lysine-utilizing DAPA aminotransferase." (*Id.* at 8).

With a view towards furthering prosecution and as suggested by the Examiner, claims 1-4 have been amended to recite --*Bacillus subtilis* lysine-utilizing DAPA aminotransferase--. Accordingly, this rejection is rendered moot and should be withdrawn.

2. Enablement

Claims 1-22 were rejected under 35 U.S.C. § 112, first paragraph. (Paper No. 35 at 8). In making the rejection, the Examiner asserted that "the specification... does not reasonably provide enablement for practicing the claimed method with a bacterial cell comprising any lysine-utilizing DAPA aminotransferase." (*Id.*).

The Examiner acknowledged, however, that the specification is "enabling for a method for the production of biotin vitamers using a bacterial cell comprising *B.*

subtilis lysine-utilizing DAPA aminotransferases and wherein the lysine or biotin synthesis in said bacterial cell is deregulated by mutations in the genes encoding aspartokinase, I, II, III or DAP decarboxylase." (*Id.*).

The Examiner further indicated that "the instant rejection may be overcome by limiting the claims to a *B. subtilis* lysine-utilizing DAPA aminotransferase." (*Id.* at 11).

With a view towards furthering prosecution and as suggested by the Examiner, claims 1-4 have been amended to recite --*Bacillus subtilis* lysine-utilizing DAPA aminotransferase--. Accordingly, this rejection is rendered moot and should be withdrawn.

In view of the agreement reached with the Examiner during the Interviews, favorable action on the merits, including entry of the amendments, withdrawal of the rejections and objections, and allowance of all the claims, respectfully are requested. If the Examiner has any questions regarding this paper, please contact the undersigned attorney.

Respectfully submitted,

By: _____

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I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on August 22, 2003.

_____

Gonzalo Merino, Ph.D., Reg. No. 51,192

TABLES

FOR

UNITED STATES LETTERS PATENT

TITLE: OVERCOMING DAPA AMINOTRANSFERASE BOTTLENECKS IN BIOTIN VITAMERS BIOSYNTHESIS

APPLICANT: SCOTT W. VAN ARSDELL, R. ROGERS YOCUM, JOHN B. PERKINS, and JANICE G. PERO

9 PAGES OF TABLES

TABLE 1

Amino donor tested	Stimulation of activity	Amino donor tested	Stimulation of activity
none	–	L-glutamic acid	–
L-methionine	–	L-lysine	+
L-aspartic acid	–	L-tryptophan	–
L-asparagine	–	L-valine	–
L-tyrosine	–	L-leucine	–
L-cysteine	–	L-alanine	–
L-proline	–	L-isoleucine	–
L-serine	–	L-ornithine	–
L-glycine	–	L-homoserine	–
L-glutamine	–	DL-homocysteine	–
L-threonine	–	spermine	–
L-histidine	–	S-adenosyl-L-methionine	–
L-phenylalanine	–	S-adenosyl-L-homocysteine	–
L-arginine	–		

TABLE 2

Compound added to extract	DAPA aminotransferase specific activity (nmoles/min/mg)
none	0
L-lysine (>98%)	0.76
L-lysine (>99%)	0.56
D-lysine (>98%)	0.19
DL-lysine (>98%)	0.35
Nα-acetyl-L-lysine	0
Nε-acetyl-L-lysine	0
Nε-methyl-L-lysine	0
gly-lys	0
lys-gly	0
(S)-2-aminoethyl-L-cysteine	0.48
diaminopimelic acid	0

TABLE 3

Fermentation #/ Strain	Lysine (6 g/liter) Batch	Feed	Time (hr)	OD_{600}	Total Vitamers (mg/liter)	Biotin (mg/liter)	HABA Vitamers (mg/liter)	Calculated DTB (mg/liter)
B160/BI603	+	–	24	150	740	16	330	314
B160/BI603	+	–	30	160	950	22	400	378
B161/BI603	+	+	24	140	1100	14	420	406
B161/BI603	+	+	30	160	1290	20	570	550
B162/BI282	+	+	24	132	1100	10	220	210
B162/BI282	+	+	30	140	1000	22	330	308

Vitamer Breakdown

Fermentation #/ Strain	Lysine (6 g/liter) Batch	Feed	Time (hr)	KAPA (mg/liter)	DAPA[a] (mg/liter)	DTB (mg/liter)	Biotin (mg/liter)	Total (mg/liter)
B161/BI603	+	+	30	710	10	550	20	1290

[a] Estimated from bioautography of a an acid autoclaved sample using *E. coli* MEC1 indicator.

TABLE 4

Fermentation #/ Strain	Time (hr.)	OD_{600}	Total Vitamers (mg/liter)	Biotin (mg/liter)	HABA Vitamers (mg/liter)	Calculated DTB (mg/liter)
BI63/BI90	24	150	760	8	126	118
BI63/BI90	30	160	720	9	145	136
BI64/BI96	24	170	830	9	84	75
BI64/BI96	30	160	850	10	88	78
B165/BI282	24	140	610	5	17	12
BI65/BI282	30	150	590	6	25	19

TABLE 5A

Fermentation #/ Strain	Batch and Feed Lys (6 g/liter)	Batch and Feed Met (3 g/liter)	Time (hr)	OD_{600}	Total Vitamers (mg/liter)	Biotin (mg/liter)	HABA Vitamers (mg/liter)	Calculated DTB (mg/liter)
B166/BI603	–	–	24	150	800	20	30	10
B166/BI603	–	–	30	155	600	21	30	9
B167/BI603	+	–	24	143	800	6	460	454
B167/BI603	+	–	30	166	870	5	510	506
B168/BI90	+	+	24	128	800	5	890	885
B168/BI90	+	+	30	165	1000	5	930	925

TABLE 5B

Vitamer Breakdown

Fermentation #/ Strain	Batch and Feed: Lys (6 g/liter)	Batch and Feed: Met (3 g/liter)	Time (hr)	KAPA (mg/liter): [a]	KAPA (mg/liter): [b]	DAPA[c] (mg/liter)	DTB (mg/liter)	Biotin (mg/liter)	Total (mg/liter)
B166/BI603	–	–	30	570	470	0	9	21	600
B167/BI603	+	–	30	320	250	40	505	5	870
B168/BI90	+	+	30	55	60	15	925	5	1000

[a] Calculated by subtracting DAPA, DTB, and biotin titers from total vitamers.

[b] Estimated from bioautography of acid autoclaved samples using *E. coli ΔbioH* indicator.

[c] Estimated from bioautography of acid autoclaved samples using *E. coli* MEC1 indicator.

TABLE 6

Run/Strain (Drug)	Lysine (g/liter) Batch	Lysine (g/liter) Feed	Time (hr.)	OD_{600}	Total Vitamers (mg/liter)	HABA Vitamers (mg/liter)	Biotin (mg/liter)	%KAPA to DTB conversion (mg/liter)
B235/B1282 (CAM60)	7.5	24.8	24	107	590	600	4	100
			30	122	830	660	4	89
B236/B1282 (CAM60)	---	---	24	123	410	40	11	10
			30	130	450	60	12	13
B237/B1282 (CAM60)	7.5	7.5	24	115	630	780	4	100
			30	124	670	750	5	100

*Batch medium (Amberex) contained 1 g/l pimelic acid and the indicated lysine amount; Feed medium contained 1 g/l pimelic acid and the indicated lysine amount.

Table 7

Enzyme	Type of Mutation	Gene	Map Location	Inhibitor	Corepressor	Decrease in stationary
Aspartokinase I	DAP^r	*dapG*	149	DAP	none known	no
Aspartokinase II	constitutive	*lysC*	252	lysine	lysine	yes
Aspartokinase III	---	---	---	lysine & threonine	threonine	yes
DAP decarboxylase	lys^r	*lysA*	210	lysine	lysine & ?	yes
---	---	*aecB*	282	---	---	---

TABLE 8

Fermentation #/ Strain	Lysine (6 g/liter) Batch	Lysine (6 g/liter) Feed	Time (hr)	OD600	Total Vitamers (mg/liter)	Biotin (mg/liter)	HABA Vitamers (mg/liter)	Calculated DTB (mg/liter)
B190/BI282	+	+	24	84	240	6	270	264
B190/BI282	+	+	30	125	390	7	360	353
B191/BI641 (BI282*aec7*)	–	–	24	74	470	5	130	125
B191/BI641 (BI282*aec7*)	–	–	30	129	500	6	144	138
B192/BI642 (BI603*aec11*)	–	–	24	86	540	4	160	156
B192/BI642 (BI603*aec11*)	–	–	30	120	560	5	110	105

APPENDICES

FOR

UNITED STATES LETTERS PATENT

TITLE: OVERCOMING DAPA AMINOTRANSFERASE BOTTLENECKS IN BIOTIN VITAMERS BIOSYNTHESIS

APPLICANT: SCOTT W. VAN ARSDELL, R. ROGERS YOCUM, JOHN B. PERKINS, and JANICE G. PERO

2 PAGES OF APPENDICES

Appendix I. Medium composition for biotin and vitamers production in bench scale fermentors.

Medium Component	Concentration	
	Batch	Feed
Glucose	15.0 g/liter	750 g/liter
Veal Infusion Broth[1]	25.0 g/liter	- - -
Yeast Extract[1]	5.0 g/liter	- - -
Sodium Glutamate	5.0 g/liter	- - -
KH_2PO_4	7.5 g/liter	13.7 g/liter
$MgCl_2 \cdot 6H_2O$	1.0 g/liter	1.5 g/liter
$(NH_4)_2SO_4$	2.0 g/liter	- - -
MAZU DF-37C	2.5 g/liter	- - -
$CaCl_2 \cdot 2H_2O$	1.0 g/liter	- - -
$CuSO_4 \cdot 5H_2O$	0.4 mg/liter	4.0 mg/liter
$ZnSO_4 \cdot 7H_2O$	0.5 mg/liter	5.0 mg/liter
$MnSO_4 \cdot H_2O$	25.0 mg/liter	35.0 mg/liter
$CoCl_2 \cdot 6H_2O$	1.0 mg/liter	10.0 mg/liter
Sodium Molybdate-$2H_2O$	0.2 mg/liter	2.0 mg/liter
$FeSO_4 \cdot 7H_2O$	50.0 mg/liter	100.0 mg/liter
Sodium Citrate-$2H_2O$	50.0 mg/liter	100.0 mg/liter

[1] In Amberex Medium the Veal Infusion Broth and Yeast Extract are replaced with 10 g/l Amberex 695.

Appendix II. Protocol of avidin-HABA [2-(4-hydroxyphenylazo) benzoic acid] displacement assay for biotin and dethiobiotin.

Reagents and Solutions:

Buffer:	0.1 M $NaPO_4$, pH 7.0.
Avidin:	From Sigma (Cat # A-9275). Dissolved at 5 mg/ml in Buffer.
HABA:	From Aldrich (Cat # 14,803-2). Dissolved at 0.375 M in water + 1 eq. NaOH.

Prepare Mix:

	20 samples		50 samples	
Avidin	1	ml	2.5	ml
HABA	0.08	ml	0.2	ml
Buffer	38.9	ml	97.3	ml

Assay:

Zero spectrophotometer;

Add 2 ml of Buffer to disposable 5 ml cuvette; record OD_{500}.

To read sample:

Place disposable 5 ml cuvette in spectrophotometer.
Add 2 ml of Mix; stir; record OD_{500}.
Add sample in 0.1 ml volume; stir; record OD_{500}.

Standards:

Use 0.1 ml DTB at 2 mg/ml to 14 mg/ml as samples.
Use 0.1 ml Buffer as "zero" point.

Calculations:

Calculate ΔOD_{500} minus ΔOD_{500}.
Plot standards and use curve to determine HABA vitamers from samples.

Notes:
1. Useful range is 2 to 14 mg/l of biotin + dethiobiotin.
2. Add mix to cuvette, read OD500, and then add sample and mix without removing cuvette from the spectrophotometer.
3. Best results are obtained when a constant volume is used with a set of samples and standards. Use Buffer to bring all samples to the same volume.

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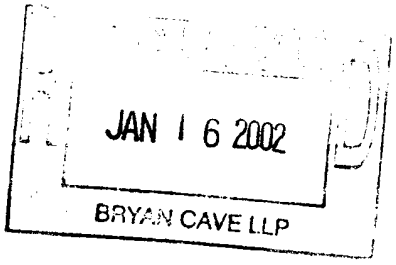

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